NONLETHAL BIRD REPELLENTS: IN SEARCH OF A GENERAL MODEL RELATING REPELLENCY AND CHEMICAL STRUCTURE

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Abstract: Identification of potential repellents through molecular modeling has implications for the development of commercially viable, ecologically sound, nonlethal bird repellents. We tested isomers (ortho, meta, para) and moieties (amino, hydroxy, methoxy) of acetophenones for their effectiveness as bird repellents to better understand the nature of repellency in birds. Chemically, basicity of a substituted phenyl ring, as defined by the electron-donating substituent, probably is an important feature influencing repellency; i.e., more basic substituents result in more potent repellents. Isomeric position of the electron-donating substituent, which leads to resonance of lone pairs of electrons, is also an important feature of repellency; i.e., repellency is enhanced when electron-donating substituents are in the ortho and para positions. An ancillary contributory factor enhancing repellency is the presence of intramolecular hydrogen bonds. Combining these features improves repellency. Thus, the most potent repellent, ortho-aminoacetophenone (OAAP), effective at concentrations ≤0.0003 vol/vol, is the most basic of the series tested. It has the amino group in the ortho position which enables this group to form a hydrogen bond with the carbonyl electron-withdrawing group.

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The ability to identify an environmentally safe avian repellent has numerous advantages, the paramount being that the birds can be kept away from crops or other areas with minimum risk to the birds. Progress has been made in applications of such repellents in agriculture (Mason et al. 1985, Glahn et al. 1989, Thompson 1989). However, application of bird repellents in nonagricultural situations is of increasing concern. The treatment of wastewater is 1 example where an effective avian repellent may be employed. The gold and silver mining industries rely on cyanide extraction techniques to obtain precious metals from ore. The resulting wastewater is held in leach fields and surface ponds until the cyanide can be recovered or quenched. Because such mines are often located in arid areas, the water represents a lethally attractive resource to resident and migrating birds. Unprotected ponds have resulted in significant kills (Allen 1990). To date, the only reliable means of preventing bird access to the ponds is use of exclusionary netting (Jackson 1990). However, the logistics of engineering netting over large areas is costly and difficult (Schroeder 1990). The use of chemical repellents to deter birds from drinking and swimming in wastewater ponds may be a viable ancillary or primary strategy in protecting wildlife from accidental ingestion of toxic and lethal wastewater.

Adding repellents to freshwater might also be desirable. Collisions between birds and aircraft incur serious economic losses and pose a significant risk to public safety (Blokpoel 1976; R. Dolbeer, U.S. Dep. Agric., Anim. and Plant Health Inspection Serv., pers. commun.). Additives to freestanding water that repel birds would be a major contribution to solving bird nuisance and safety problems at airports.

A challenge facing the application of chemical repellents is their stability under a variety of physical conditions. Repellents identified for use as feed additives might not be suitable for the caustic conditions of wastewater impoundments. Presently, the discovery of repellents is empirical and at times serendipitous (e.g., Mason et al. 1991). The heuristic modeling approach that we use might allow us to make predictions about the nature of irritation in birds, thereby leading to a more efficient method of identifying agents for use in nonlethal bird control.

A variety of esters of anthranilic acid are repellent to birds, but not to mammals; the most effective being methyl and dimethyl anthranilate (Kare 1961). Noting the perceptual similarity of methyl anthranilate to ortho-aminoacetophenone (OAAP) to humans (Acree et al. 1990), we tested OAAP as a bird repellent (Mason et al. 1991). Ortho-aminoacetophenone was a more

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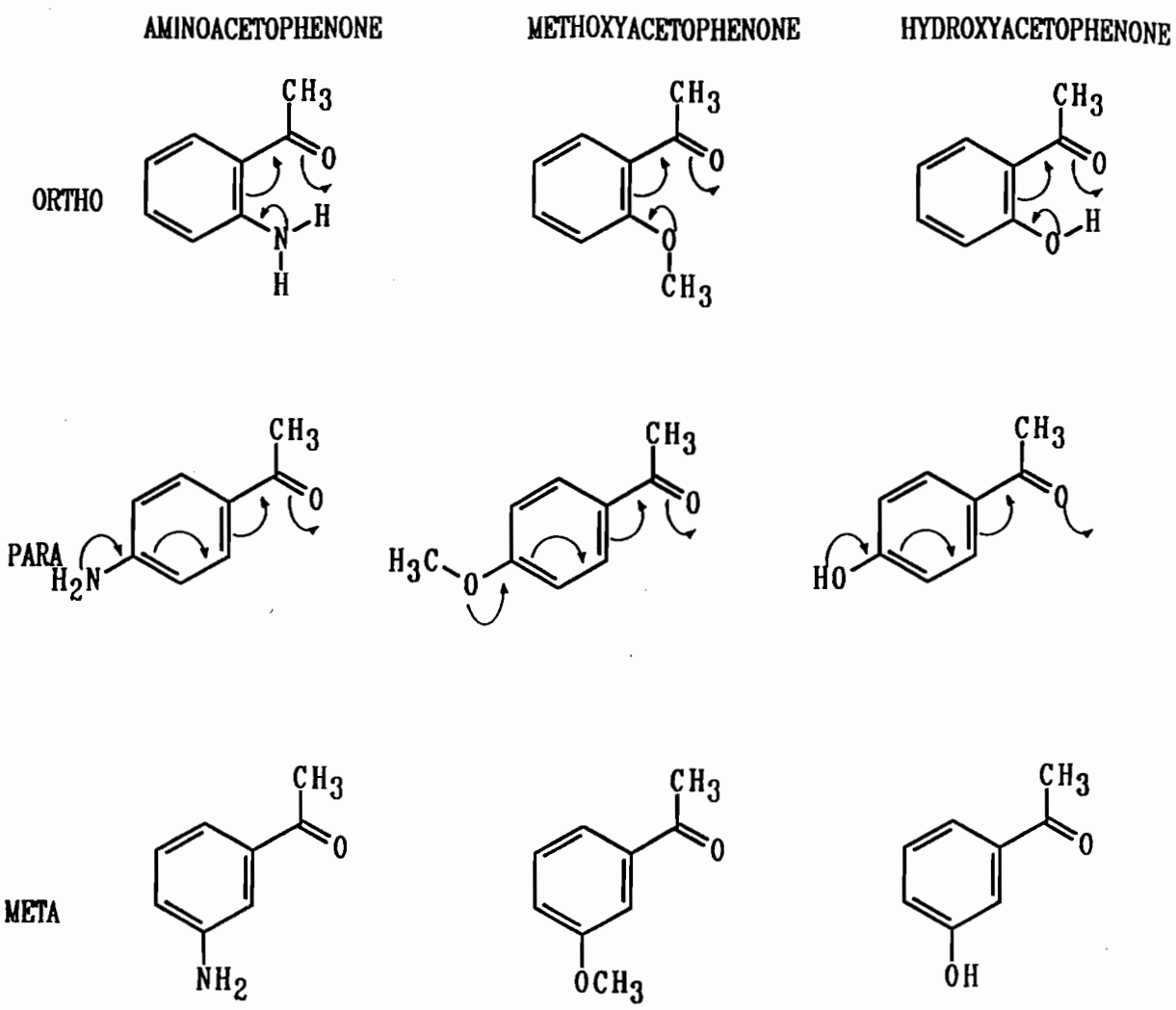

Fig. 1. Structural and resonance representation of the compounds we tested. AAP = aminoacetophenone, MOAP = methoxyacetophenone, HAP = hydroxyacetophenone, 2 = ortho, 3 = meta, 4 = para.

potent bird repellent than any of the anthranilates previously tested. Based on the relative repellencies of anthranilates (Mason et al. 1989) and aminoacetophenones (Mason et al. 1991), we hypothesized that intramolecular hydrogen bonding and delocalization of lone pairs of electrons (resonance) were important features of bird repellency.

The objective of our study was to further clarify the effects of isometric changes for moieties of acetophenones on repellency (Fig. 1). We studied amino, methoxy, and hydroxy substitutions because these configurations allowed us to systematically test for the effects of hydrogen bonding, basicity, and position (isomerization) on repellency.

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METHODS

Birds.—Adult European starlings (*Sturnus vulgaris*) were decoytrapped in rural New Jersey and transported to the laboratory. Upon arrival, the birds were individually caged (61 × 36 × 41 cm) under a 12:12 light : dark cycle with light onset at 0700 hours. Food and grit (Purina Bird Flight Conditioner and medicated oyster shells) were available ad libitum; apples were given twice a week. Birds were captured in March 1990 and held for 3 weeks under lab-

oratory conditions before testing began in mid-April. Before experiments began, the birds were permitted free access to tap water.

Chemicals.—We obtained all isomers of aminoacetophenone, hydroxyacetophenone, and methoxyacetophenone from Aldrich Chemical Company, Wisconsin. Each moiety has a phenyl ring with an electron-donating primary amino, hydroxy, or methoxy group and an electron-withdrawing carbonyl group. The strength of donation is ranked as: amino > methoxy > hydroxy. Isomers of each moiety differ only in their substitution patterns on the phenyl ring (Fig. 1). Because acetophenones are generally insoluble in water, we mixed each compound in water under low heat to yield saturated emulsions with concentrations at 0.5% (mass/vol or vol/vol). Lower concentrations were established by serial dilutions to yield 0.25, 0.125, 0.0625, and 0.0313%. Plain water was used as a control (0%); thus, 6 concentrations were tested.

One-Choice Tests.—Starlings were given 3 days of pretreatment during which water consumption was measured for 6 hours. At the end of this period, individuals whose variance about the 3-day mean consumption was > ±1 standard deviation of the population variance were excluded from the trials. Those birds with stable daily water consumption were ranked according to mean water consumption and assigned to treatment groups. The bird with the highest water consumption was assigned to the 0.5% treatment group, the bird with the second highest consumption was assigned to the 0.25% treatment group, and so forth, until all birds were assigned to a group. We used 24 birds for the experiments, i.e., 4 birds per treatment group.

After assigning birds to a treatment group, we began their 1-day drinking trial. Birds had free access to feed and tap water during night. Beginning at 0930 hours, water was replaced with preassigned concentrations of chemicals, and we recorded consumption every 2 hours for the next 6 hours. After the test, birds were given free access to tap water. Consumption of tap water was monitored overnight and for a 6-hour period the next day. We monitored overnight consumption to evaluate whether birds compensated for any water deficits resulting from the experiments. The within group, 6-hour posttreatment-day water consumption was compared with mean within group, 6-hour pretreatment water consumption to determine whether consumption had returned to pretreatment levels. If not, we postponed initiation of testing of the next compound until diurnal water consumption returned to pretreatment levels. Once the condition was met, we tested the birds with the next compound, with groups of birds receiving a different concentration of compound as determined by a counter-balanced predetermined schedule; i.e., test groups initially were randomly assigned to 1 of the 6 concentrations to be tested. On subsequent tests, groups were assigned the next highest concentration (e.g., birds initially tested at 0.6% concentration for 1 compound were subsequently tested at 1.3, 2.5, 5.0, 0, 0.3% concentrations for the remaining compounds). The order in which chemicals were tested was arbitrary: OAAP, 3AAP, 4AAP, 2MOAP, 2HAP, 4HAP, 3HAP, 3MOAP, and 4MOAP.

We did not use naive birds for each test because it was not practical to capture the 216 starlings required for all experiments. If an individual's 6-hour interexperiment water consumption was within ±1 standard error of its pretreatment value, the bird was used in the next experiment (i.e., testing of the next compound), otherwise we substituted a new bird. Throughout the experiment only 2 birds were replaced. Replacement was necessary because these birds developed the habit of jumping up and down on the drinking tubes, thus causing spillage. In addition, we checked birds for health condition after each experiment. There were no mortalities, and at the end of all experiments the birds were released.

Analyses.—To test for carry-over effects due to treatment, we examined the intertreatment tap water consumption of all birds with a repeated measures, 1-way ANOVA. We compared each posttreatment day's water consumption by partition using an a priori contrast with pretreatment water consumption (Norusis 1986). We considered each isomeric moiety separately. The experimental design precluded the testing for order effects of the chemical compounds.

We tested 2 a priori hypotheses about consumption of treated water. First, did consumption of treated water differ from a theoretical value of zero consumption? This information is useful because there may be times when a bird must be repelled from potentially lethal toxic wastewater, e.g., cyanide ponds resulting from gold mining. The analysis required a slight modification in calculation of the treatment sums of squares, where the grand mean was replaced by

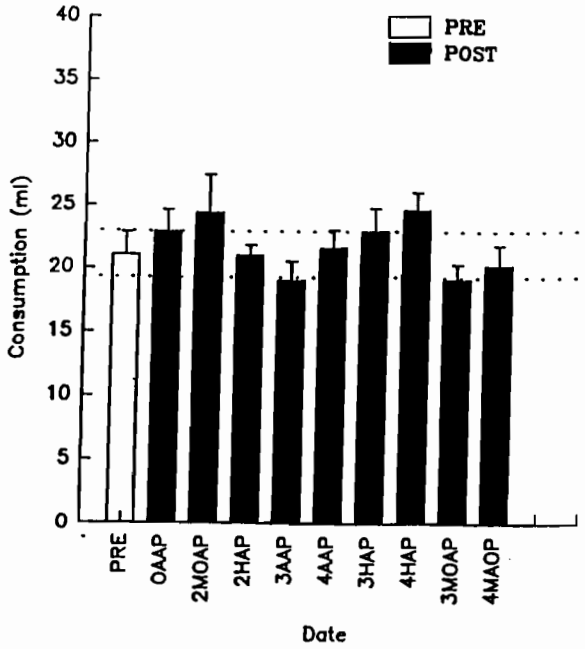

Fig. 2. Tap water consumption recorded over 6 hours for the day following treated water tests. Pretreatment levels (open bar) were compared to posttreatment level of consumption (solid bars). Error bars depict 1 standard error. Horizontal lines depict ±1 standard error of pretreatment water consumption. See Figure 1 for chemical codes.

zero and the degrees of freedom reflected the number of treatments (6) considered in the experiment. Estimates of the error term remained the same as in a standard ANOVA. We made post hoc comparisons with a modification of Dunnett's *t*-test (1955), again using a theoretical value of zero rather than the mean, and comparing the resulting *t* to critical values in Dunnett's calculated distribution with *P* set at <0.05. Second, did mean water consumption differ among the treatment (concentration) groups? We used a 1-way ANOVA to compare group means and a Scheffe's post hoc test to identify significant ($P < 0.05$) differences among means.

We used a least-squares fit for a nonlinear function of the form $y(x) = a(\exp - sx)$ with the Marquardt–Levenberg algorithm (Jandel Sci. 1989), where *a* is the consumption at 0% concentration, *s* is the rate of change, and *x* is the concentration of the compound, to fit curves for the dose–response relationship. We used the 2-parameter model because it provided the best fit for most of the compounds tested in our laboratory over the broadest range of concentrations.

We restricted our analysis to a heuristic evaluation of the importance of basicity, resonance, and intramolecular hydrogen bonding by comparing ranked water consumption as a function of these chemical attributes. General contributions of attributes to repellency were determined by calculating mean consumption across all concentrations relative to the plain water control. Thus, for the ortho isomeric position, we calculated relative consumption by taking the average of the relative mean consumptions of OAAP, 2MOAP, and 2HAP. The relative consumptions for meta (3AAP, 3MOAP, 3HAP) and para (4AAP, 4MOAP, 4HAP) isomers were calculated similarly. To rank compounds for basicity, relative consumptions were calculated in similar fashion for the amino (OAAP, 3AAP, 4AAP), methoxy (2MOAP, 3MOAP, 4MOAP), and hydroxy (2HAP, 3HAP, 4HAP) groups. Calculations for hydrogen bonding were similar, with the grouping consisting of bond present (OAAP, 2HAP) and absent (3AAP, 4AAP, 2MOAP, 3MOAP, 4MOAP, 3HAP, 4HAP).

RESULTS

On 3 occasions, the starlings' posttreatment day consumption of tap water differed significantly from the pretreatment levels (Fig. 2). However, there was no pattern to indicate that changes in diurnal posttreatment water consumption resulted from treatment of the water with repellent. In the case of OAAP and 4HAP treatments, the posttreatment water consumptions exceeded the pretreatment levels ($P = 0.021$ and <0.001, respectively); OAAP was a good repellent, whereas 4HAP was not repellent. In the latter case, it is unlikely that the increased posttreatment consumption reflected compensation because water consumption during treatment did not differ from control levels. Posttreatment consumption was lower than pretreatment levels for 4MOAP ($P = 0.002$). In general, there was no apparent relationship between the effectiveness of a repellent and water consumption recorded during the 6-hour trial during the following posttreatment day.

Within Compound Comparison Among Concentrations.—Within the hydroxy series, only 2HAP showed any signs of repellency (Fig. 3), and then only the highest concentration (0.5%) was different from the control (Table 1). the other 2 compounds, 3HAP and 4HAP, were totally ineffective as repellents. All 3 isomers of the methoxy series showed some signs of repellency (Fig. 3). In general, only the highest concentration (0.5%) was different from the control, though the para isomer was effective at concentrations as low as 0.13% (Table 1). The iso-

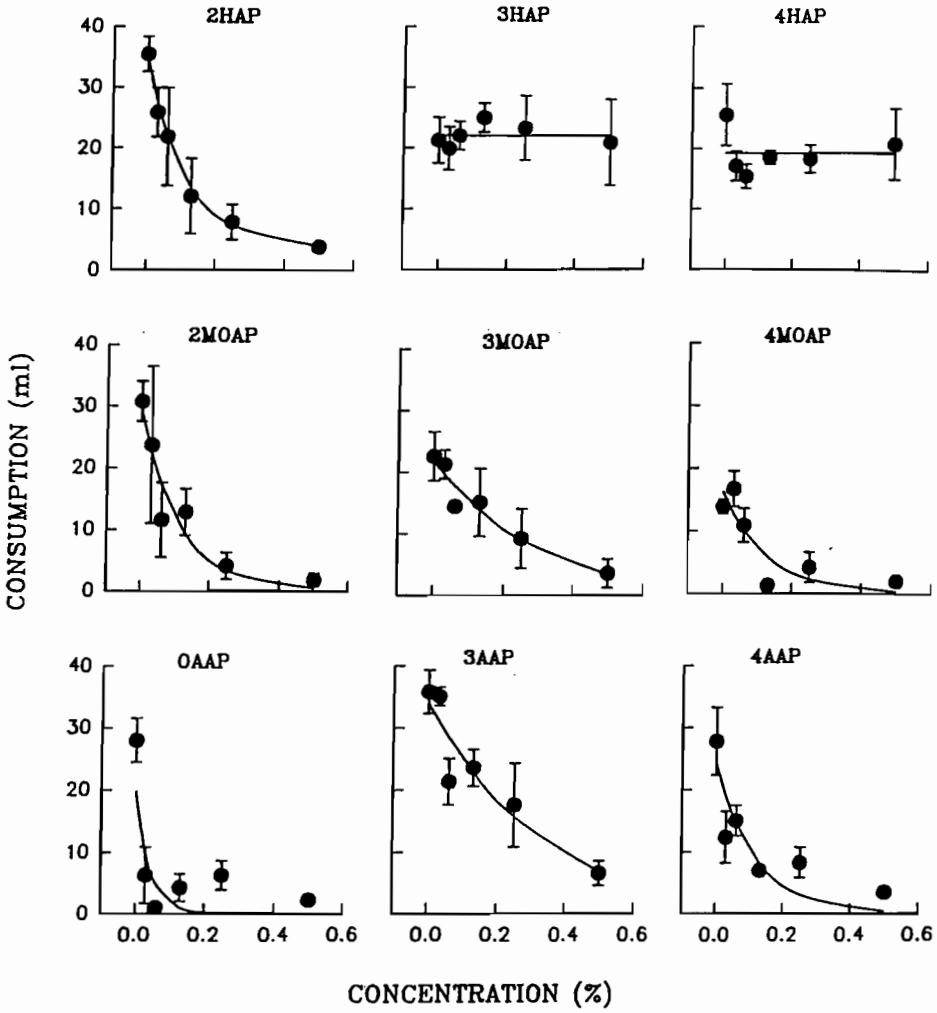

Fig. 3. Dose–response curves for the acetophenone series we tested. Points represent mean consumption for 4 birds per group ±1 standard error. See Figure 1 for chemical codes.

mers of the amino series were uniformly good repellents (Fig. 3). All concentrations of OAAP differed from the control; whereas 4AAP differed down to a concentration of 0.13%. The meta isomer was only effective down to a concentration of 0.5% (Table 1).

Within Compound Comparison to Zero Consumption.—Not all compounds were

Table 1. Differences in mean water consumption among concentrations tested.

Compound[a]	F[b]	P[c]	Ranked ordered concentrations[d]
OAAP	11.30	0.001	0.06 0.50 0.13 0.03 0.25 0
3AAP	7.53	0.001	0.50 0.25 0.13 0.06 0.03 0
4AAP	7.69	0.001	0.50 0.13 0.25 0.06 0.03 0
2HAP	5.32	0.004	0.50 0.25 0.13 0.06 0.03 0
3HAP	0.17	0.970	0.50 0.25 0.13 0.06 0.03 0
4HAP	0.95	0.463	0.50 0.25 0.13 0.06 0.03 0
2MOAP	4.58	0.007	0.50 0.25 0.06 0.13 0.03 0
3MOAP	3.74	0.009	0.50 0.25 0.06 0.13 0.03 0
4MOAP	10.89	0.001	0.13 0.50 0.25 0.06 0.03 0

[a] See Figure 1 for chemical codes.
[b] F-values of the ANOVA for concentration effect (5,18 df). Each concentration group consisted of 4 birds.
[c] P reports the exact probability values of the F-test.
[d] Rank order of concentrations based upon mean consumption. Concentrations connected with the same line are not different ($P > 0.05$).

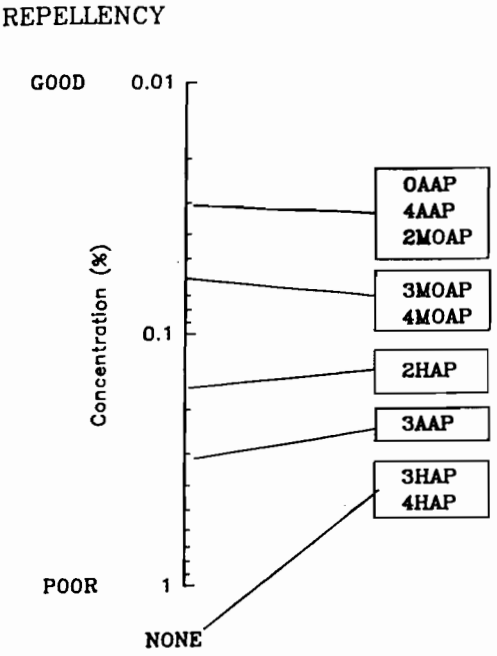

Fig. 4. Ranking of repellency for compounds we tested. Ranking shown is the lowest concentration tested where consumption did not differ from zero consumption, i.e., the lowest concentration of absolute repellency.

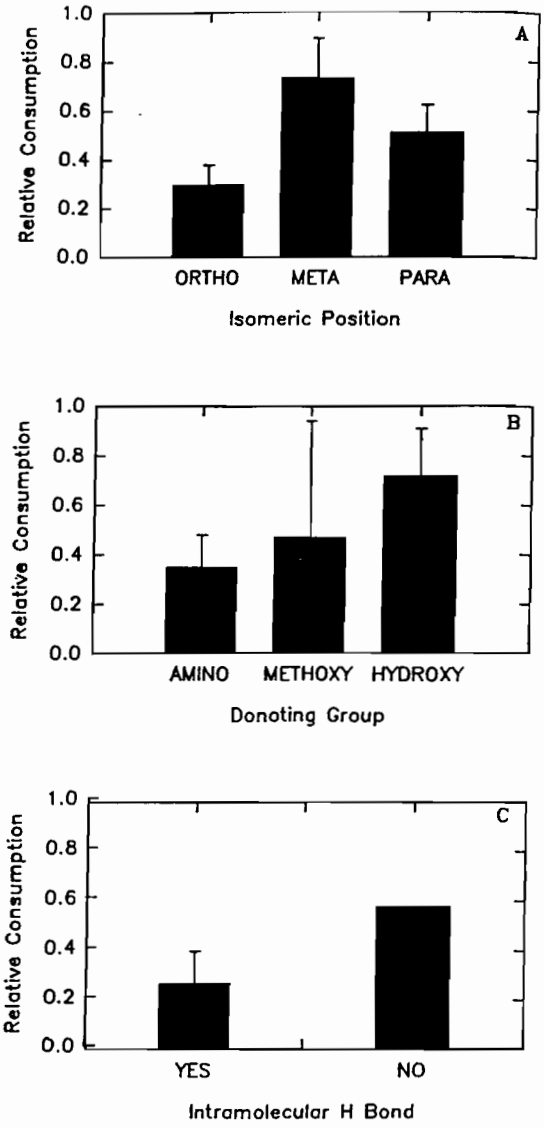

Fig. 5. A. Ranking of relative water consumption as a function of isomeric position of the electron-donating group. Ortho and para isomers are capable of resonance of lone electron pairs, the meta isomers are not. Consumption is relative to consumption of water controls. B. Ranking of relative water consumption as a function of basicity of the molecule. Amino substituents are the most basic; hydroxy substituents are the least basic. C. Ranking of relative water consumption as a function of presence or absence of intramolecular hydrogen (H) bonding.

equally effective at deterring birds from drinking water (Fig. 4). Two compounds, 3HAP and 4HAP, were totally ineffective as repellents; OAAP showed absolute repellency over the broadest range of concentrations tested. All concentrations were equivilent to zero consumption, suggesting that even lower concentrations might be effective absolute repellents. Compounds 4AAP and 2MOAP were also effective absolute repellents, but they probably are not as effective as OAAP (Fig. 3). The remaining compounds were less effective as absolute repellents at low concentrations (Fig. 4).

Contribution of Chemical Attribute to Repellency.—Molecules with substituents in the meta position were the least effective repellents (Fig. 5A). The isomers whose lone pairs of electrons were in resonance with the carbonyl group (ortho and para) were better repellents. In addition, the more basic compounds were the more effective repellents (Fig. 5B). Finally, the 2 compounds capable of intramolecular hydrogen bonding were better repellents than molecules incapable of such bonds (Fig. 5C).

DISCUSSION

There was no indication that consumption of repellent affected long-term, posttest water consumption. In the 3 trials where consumption of tap water differed from pretreatment levels, consumption returned to normal the following day. Any potential deficits in water consumption resulting from repellency were generally made up within the first few hours after completion of the trial, i.e., overnight. Furthermore, there was no indication that any of the compounds had toxic effects, such as piloerection, that might affect subsequent water consumption. Because there was no relationship between posttreatment water consumption and magnitude of repellency, we believe that any short-term differences in water consumption over time

merely reflected intrinsic variation in consumption levels. Our experimental design did not permit us to directly assess the effect of the order of chemical presentation. However, combined with the lack of carry-over effect on posttreatment water consumption and the apparent lack of a relationship between the magnitude of repellency for consecutive treatment trials, we conclude that any potential order effect attributable to learning is unlikely.

We previously suggested that intramolecular hydrogen-bonding capacities and resonance were associated with strength of repellency (Mason et al. 1991). Intramolecular hydrogen bonding probably is not a primary feature for repellency because (1) intramolecular hydrogen bonding is only possible in the ortho isomers of aminoacetophenone and hydroxyacetophenone, yet meta and para isomers of aminoacetophenone were also good repellents, and (2) the isomers of methoxyacetophenone are incapable of forming intramolecular hydrogen bonds, yet these structures are as good or better repellents than the ortho isomer of hydroxyacetophenone where intramolecular hydrogen bonding is possible. The diminution of the importance of hydrogen bonding in drinking trials may be due to the possible solvation of the polar groups (OCH_3 and NH_2 or OH) by water molecules which can make the intramolecular hydrogen bonding irrelevant in this medium.

The strength of repellency decreases as a function of the positional isomer, e.g., ortho isomers are better repellents than para isomers, which in turn are better than meta isomers, suggesting that electron donation by resonance is an important feature of repellency (Fig. 5). The ortho and para isomers are in direct resonance with the carbonyl group, whereas the meta isomer is not (Fig. 1). The meta isomers will actually withdraw the electrons from the phenyl ring through the inductive effect.

We found that basicity (and/or electron-donating ability of the substituent group) is an important factor controlling repellency (Fig. 5). The relative basicity for the compounds is greatest for amino substitutions and least for hydroxy substitutions, a ranking concordant with the data on strength of repellency.

Interaction effects are undoubtedly important. For example, the most effective repellent by any measure is OAAP. This molecule is the most basic and has its amino group in the ortho position. As a consequence, the amino group is in a position to form an intramolecular hydrogen bond with the electron-withdrawing group, suggesting an ancillary contributory role to repellency of such bonding. Intramolecular hydrogen bonding is not crucial to repellency because the basic para and meta isomers (4AAP, 4MOAP; and 3AAP, 3MOAP, respectively) are effective repellents. Basicity, and not resonance, may be the most important feature of repellency. The relatively acidic para isomer of hydroxyacetophenone, which is capable of resonance, is an ineffective repellent. Only when the substituent is in the ortho position does this hydroxyacetophenone become repellent. This suggests that intramolecular hydrogen bonding interacts with resonance to overcome the negative effects of the relative acidity on repellency.

To better understand the contribution of resonance versus basicity for repellency, we are looking into an array of stimuli, e.g., anisoles and anthranilate derivatives, where these variables can be independently and systematically manipulated. Nonetheless, we suggest that structure–activity relationships could indeed be useful for constructing general models for avian repellents.

LITERATURE CITED

ACREE, T. E., E. H. LAVIN, R. NISHIDA, AND S. WATANABE. 1990. The serendipitous discovery of ortho-aminoacetophenone as the 'foxy' smelling component of Labruscana grapes. Chem. and Eng. News 9:80.

ALLEN, C. 1990. Mitigating impacts to wildlife at FMC Gold Company's Paradise Peak Mine. Pages 67–71 *in* R. McQuivey, coord. Proc. Nevada wildlife/mining workshop. Nev. Mining Assoc., Nev. Dep. Minerals, and Nev. Dep. Wildl., Reno.

BLOKPOEL, H. 1976. Birds hazards to aircraft. Can. Wildl. Serv., Ottawa. 236pp.

DUNNETT, C. W. 1955. A multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. 50:1096–1121.

GLAHN, J. F., J. R. MASON, AND D. R. WOODS. 1989. Dimethyl anthranilate as a bird repellent in livestock feed. Wildl. Soc. Bull. 17:313–320.

JACKSON, W. B. 1990. Bird repelling techniques. Pages 46–50 *in* R. McQuivey, coord. Proc. Nevada wildlife/mining workshop. Nev. Mining Assoc., Nev. Dep. Minerals, and Nev. Dep. Wildl., Reno.

JANDEL SCIENTIFIC. 1989. Sigmaplot, scientific graph system, v.4.0. Corte Madra, Calif. 204pp.

KARE, M. R. 1961. Bird repellent. U.S. Patent 2,967,128.

MASON, J. R., M. A. ADAMS, AND L. CLARK. 1989. Anthranilate repellency to starlings: chemical correlates and sensory perception. J. Wildl. Manage. 53:55–64.

———, L. CLARK, AND P. S. SHAH. 1991. Orthoaminoacetophenone repellency to birds: similarities to methyl anthranilate. J. Wildl. Manage. 55:334–340.

———, J. F. GLAHN, R. A. DOLBEER, AND R. F. REIDINGER. 1985. Field evaluation of dimethyl anthranilate as a bird repellent livestock feed additive. J. Wildl. Manage. 49:636–642.

NORUSIS, M. J. 1986. Advanced statistics, SPSS/PC+. SPSS, Inc., Chicago, Ill. 294pp.

SCHROEDER, M. L. 1990. The netting of cyanide ponds at Copperstone Gold. Pages 72–81 *in* R. McQuivey, coord. Proc. Nevada wildlife/mining workshop. Nev. Mining Assoc., Nev. Dep. Minerals, and Nev. Dep. Wildl., Reno.

THOMPSON, W. T. 1989. Agricultural chemicals III—fumigants, growth regulators, repellents, and rodenticides. Thompson Publ., Fresno, Calif. 210pp.

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